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(54) Title: RECEPTOR ACTIVATOR OF NF–KAPPA B, RECEPTOR IS MEMBER OF TNF RECEPTOR SUPERFAMILY

(57) Abstract

Isolated receptors, DNAs encoding such receptors, and pharmaceutical compositions made therefrom, are disclosed. The isolated receptors can be used to regulate an immune response. The receptors are also useful in screening for inhibitors thereof.

TITLE

RECEPTOR ACTIVATOR OF NF-KAPPA B, RECEPTOR IS MEMBER OF TNF RECEPTOR SUPERFAMILY

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokine receptors, and more specifically to cytokine receptor/ligand pairs having immunoregulatory activity.

BACKGROUND OF THE INVENTION

Efficient functioning of the immune system requires a fine balance between cell proliferation and differentiation and cell death, to ensure that the immune system is capable of reacting to foreign, but not self antigens. Integral to the process of regulating the immune and inflammatory response are various members of the Tumor Necrosis Factor (TNF) Receptor/Nerve Growth Factor Receptor superfamily (Smith et al., *Science* 248:1019; 1990). This family of receptors includes two different TNF receptors (Type I and Type II; Smith et al., *supra*; and Schall et al., *Cell* 61:361, 1990), nerve growth factor receptor (Johnson et al., *Cell* 47:545, 1986), B cell antigen CD40 (Stamenkovic et al., *EMBO J.* 8:1403, 1989), CD27 (Camerini et al., *J. Immunol.* 147:3165, 1991), CD30 (Durkop et al., *Cell* 68:421, 1992), T cell antigen OX40 (Mallett et al., *EMBO J.* 9:1063, 1990), human *Fas* antigen (Itoh et al., *Cell* 66:233, 1991), murine 4-1BB receptor (Kwon et al., *Proc. Natl. Acad. Sci. USA* 86:1963, 1989) and a receptor referred to as Apoptosis-Inducing Receptor (AIR; USSN 08/720,864, filed October 4, 1996).

CD40 is a receptor present on B lymphocytes, epithelial cells and some carcinoma cell lines that interacts with a ligand found on activated T cells, CD40L (USSN 08/249,189, filed May 24, 1994). The interaction of this ligand/receptor pair is essential for both the cellular and humoral immune response. Signal transduction via CD40 is mediated through the association of the cytoplasmic domain of this molecule with members of the TNF receptor-associated factors (TRAFs; Baker and Reddy, *Oncogene* 12:1, 1996). It has recently been found that mice that are defective in TRAF3 expression due to a targeted disruption in the gene encoding TRAF3 appear normal at birth but develop progressive hypoglycemia and depletion of peripheral white cells, and die by about ten days of age (Xu et al., *Immunity* 5:407, 1996). The immune responses of chimeric mice reconstituted with TRAF3$^{-/-}$ fetal liver cells resemble those of CD40-deficient mice, although TRAF3$^{-/-}$ B cells appear to be functionally normal.

The critical role of TRAF3 in signal transduction may be in its interaction with one of the other members of the TNF receptor superfamily, for example, CD30 or CD27, which are present on T cells. Alternatively, there may be other, as yet unidentified

members of this family of receptors that interact with TRAF3 and play an important role in postnatal development as well as in the development of a competent immune system. Identifying additional members of the TNF receptor superfamily would provide an additional means of regulating the immune and inflammatory response, as well as potentially providing further insight into post-natal development in mammals.

SUMMARY OF THE INVENTION

The present invention provides a novel receptor, referred to as RANK (for receptor activator of NF-κB), that is a member of the TNF receptor superfamily. RANK is a Type I transmembrane protein having 616 amino acid residues that interacts with TRAF3. Triggering of RANK by over-expression, co-expression of RANK and membrane bound RANK ligand (RANKL), and with addition of soluble RANKL or agonistic antibodies to RANK results in the upregulation of the transcription factor NF-κB, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

Soluble forms of the receptor can be prepared and used to interfere with signal transduction through membrane-bound RANK, and hence upregulation of NF-κB; accordingly, pharmaceutical compositions comprising soluble forms of the novel receptor are also provided. Inhibition of NF-κB by RANK antagonists may be useful in ameliorating negative effects of an inflammatory response that result from triggering of RANK, for example in treating toxic shock or sepsis, graft-versus-host reactions, or acute inflammatory reactions. Soluble forms of the receptor will also be useful in vitro to screen for agonists or antagonists of RANK activity.

The cytoplasmic domain of RANK will be useful in developing assays for inhibitors of signal transduction, for example, for screening for molecules that inhibit interaction of RANK with TRAF2 or TRAF3. Deleted forms and fusion proteins comprising the novel receptor are also disclosed.

The present invention also identifies a counterstructure, or ligand, for RANK, referred to as RANKL. RANKL is a Type 2 transmembrane protein with an intracellular domain of less than about 50 amino acids, a transmembrane domain and an extracellular domain of from about 240 to 250 amino acids. Similar to other members of the TNF family to which it belongs, RANKL has a 'spacer' region between the transmembrane domain and the receptor binding domain that is not necessary for receptor binding. Accordingly, soluble forms of RANKL can comprise the entire extracellular domain or fragments thereof that include the receptor binding region.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

transduced to a cell via coordination of various signaling events. Thus, a signal transduced through one member of this family may be proliferative, differentiative or apoptotic, depending on other signals being transduced to the cell, and/or the state of differentiation of the cell. Such exquisite regulation of this proliferative/apoptotic pathway is necessary to develop and maintain protection against pathogens; imbalances can result in autoimmune disease.

RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response.

Moreover, the post-natal lethality of mice having a targeted disruption of the TRAF3 gene demonstrates the importance of this molecule not only in the immune response but in development. The isolation of RANK, as a protein that associates with TRAF3, and its ligand will allow further definition of this signaling pathway, and development of diagnostic and therapeutic modalities for use in the area of autoimmune and/or inflammatory disease.

DNAs, Proteins and Analogs

The present invention provides isolated RANK polypeptides and analogs (or muteins) thereof having an activity exhibited by the native molecule (i.e, RANK muteins that bind specifically to a RANK ligand expressed on cells or immobilized on a surface or to RANK-specific antibodies; soluble forms thereof that inhibit RANK ligand-induced signaling through RANK). Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of RANK within the scope of the invention also include various structural forms of the primary proteins which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a RANK protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Derivatives of RANK may also be obtained by the action of cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and

fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α-factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of RANK proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli.*

Fusion proteins further comprise the amino acid sequence of a RANK linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG_1 having a nucleotide an amino acid sequence set forth in SEQ ID NO:8. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to FcγRI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that $Leu_{(234)}$ and $Leu_{(235)}$were critical to high affinity binding of IgG_3 to FcγRI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG_1 Fc region to decrease the affinity of IgG_1 for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANK regions.

In another embodiment, RANK proteins further comprise an oligomerizing peptide such as a leucine zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos*

(*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 1992). None of the mutations affected the ability of MVF to form a tetramer.

Amino acid substitutions in the *a* and *d* residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, CA). When all residues at position *a* are changed to isoleucine, the leucine zipper still forms a parallel dimer. When, in addition to this change, all leucine residues at position *d* are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all amino acids at position *d* with isoleucine and at position *a* with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains.

Also included within the scope of the invention are fragments or derivatives of the intracellular domain of RANK. Such fragments are prepared by any of the herein-mentioned techniques, and include peptides that are identical to the cytoplasmic domain of RANK as shown in SEQ ID NO:5, or of murine RANK as shown in SEQ ID NO:15, and those that comprise a portion of the cytoplasmic region. All techniques used in preparing soluble forms may also be used in preparing fragments or analogs of the cytoplasmic domain (i.e., RT-PCR techniques or use of selected restriction enzymes to prepare truncations). DNAs encoding all or a fragment of the intracytoplasmic domain will be useful in identifying other proteins that are associated with RANK signalling, for example using the immunoprecipitation techniques described herein, or another technique such as a yeast two-hybrid system (Rothe et al., supra).

The present invention also includes RANK with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of RANK protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A_1-Z, where A_1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z,

tertiary structure of the native protein. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Mutations in nucleotide sequences constructed for expression of analog proteins or fragments thereof must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the mRNA.

Not all mutations in the nucleotide sequence which encodes a RANK protein or fragments thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants, random mutagenesis may be conducted and the expressed mutated proteins screened for the desired activity. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene 42*:133, 1986); Bauer et al. (*Gene 37*:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent NOs. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

Other embodiments of the inventive proteins include RANK polypeptides encoded by DNAs capable of hybridizing to the DNA of SEQ ID NO:6 under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANK, or more preferably under stringent conditions (for example, hybridization in 6 X SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are

Other useful fragments of the RANK nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target RANK mRNA (sense) or RANK DNA (antisense) sequences. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

Uses of DNAs, Proteins and Analogs

The RANK DNAs, proteins and analogs described herein will have numerous uses, including the preparation of pharmaceutical compositions. For example, soluble forms of RANK will be useful as antagonists of RANK-mediated NF-κB activation, as well as to inhibit transduction of a signal via RANK. RANK compositions (both protein and DNAs) will also be useful in development of both agonistic and antagonistic antibodies to RANK. The inventive DNAs are useful for the expression of recombinant proteins, and as probes for analysis (either quantitative or qualitative) of the presence or distribution of RANK transcripts.

The inventive proteins will also be useful in preparing kits that are used to detect soluble RANK or RANKL, or monitor RANK-related activity, for example, in patient specimens. RANK proteins will also find uses in monitoring RANK-related activity in other samples or compositions, as is necessary when screening for antagonists or mimetics of this activity (for example, peptides or small molecules that inhibit or mimic, respectively, the interaction). A variety of assay formats are useful in such kits, including (but not limited to) ELISA, dot blot, solid phase binding assays (such as those using a biosensor), rapid format assays and bioassays.

The purified RANK according to the invention will facilitate the discovery of inhibitors of RANK, and thus, inhibitors of an inflammatory response (via inhibition of NF-κB activation). The use of a purified RANK polypeptide in the screening for potential inhibitors is important and can virtually eliminate the possibility of interfering reactions with contaminants. Such a screening assay can utilize either the extracellular domain of RANK, the intracellular domain, or a fragment of either of these polypeptides. Detecting the inhibiting activity of a molecule would typically involve use of a soluble form of RANK derived from the extracellular domain in a screening assay to detect molecules capable of binding RANK and inhibiting binding of, for example, an agonistic antibody or RANKL, or using a polypeptide derived from the intracellular domain in an assay to detect inhibition of the interaction of RANK and other, intracellular proteins involved in signal transduction.

Moreover, in vitro systems can be used to ascertain the ability of molecules to antagonize or agonize RANK activity. Included in such methods are uses of RANK chimeras, for example, a chimera of the RANK intracellular domain and an extracellular

sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA sequences encoding RANK, or homologs or analogs thereof which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene 2*:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β-lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature 275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149, 1968; and Holland et al., *Biochem. 17*:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α-factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem. 258*:2674, 1982) and Beier et al. (*Nature 300*:724, 1982). The yeast α-factor leader, which directs secretion

Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may express the desired protein (RANK, or homologs or analogs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

Suitable host cells for expression of proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus* spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce proteins using RNAs derived from the DNA constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of RANK, or homologs or analogs thereof that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Recombinant RANK may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then

matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANK and homologs thereof. For example, a RANK expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANK protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the RANK protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANK.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANK composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* *296*:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

The example describes the identification and isolation of a DNA encoding a novel member of the TNF receptor superfamily. A partial cDNA insert with a predicted open reading frame having some similarity to CD40 (a cell-surface antigen present on the surface of both normal and neoplastic human B cells that has been shown to play an important role in B-cell proliferation and differentiation; Stamenkovic et al., *EMBO J.* 8:1403, 1989), was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was excised from the vector by restriction endonuclease digestion, gel purified. labeled with ^{32}P, and used to hybridize to colony blots generated from a DC cDNA library containing larger cDNA inserts using high stringency hybridization and washing techniques (hybridization in 5xSSC, 50% formamide at 42°C overnight, washing in 0.5xSSC at 63°C); other suitable high stringency conditions are disclosed in Sambrook et al. in Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 1989), 9.52-9.55. Initial experiments yielded a clone referred to as 9D-8A (SEQ ID NO:1); subsequent analysis indicated that this clone contained all but the extreme 5' end of a novel cDNA, with predicted intron sequence at the extreme 5' end (nucleotides 1-92 of SEQ ID NO:1). Additional colony hybridizations were performed, and a second clone was isolated. The second clone, referred to as 9D-15C (SEQ ID NO:3), contained the 5' end without intron interruption but not the full 3'end. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

The encoded protein was designated RANK, for receptor activator of NF-κB. The cDNA encodes a predicted Type 1 transmembrane protein having 616 amino acid residues, with a predicted 24 amino acid signal sequence (the computer predicted cleavage site is after Leu24), a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail. The extracellular region of RANK displayed significant amino acid homology (38.5% identity, 52.3% similarity) to CD40. A cloning vector (pBluescriptSK-) containing human RANK sequence, designated pBluescript:huRANK (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98285.

ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:6, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 3

This example illustrates the preparation of monoclonal antibodies against RANK. Preparations of purified recombinant RANK, for example, or transfected cells expressing high levels of RANK, are employed to generate monoclonal antibodies against RANK using conventional techniques, such as those disclosed in U.S. Patent 4,411,993. DNA encoding RANK can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANK-induced signaling (antagonistic or blocking antibodies) or in inducing a signal by cross-linking RANK (agonistic antibodies), as components of diagnostic or research assays for RANK or RANK activity, or in affinity purification of RANK.

To immunize rodents, RANK immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intamuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANK, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Patent 4,703,004. A preferred screening

EXAMPLE 5

This example describes a gene promoter/reporter system based on the human Interleukin-8 (IL-8) promoter used to analyze the activation of gene transcription in vivo. The induction of human IL-8 gene transcription by the cytokines Interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF-α) is known to be dependent upon intact NF-κB and NF-IL-6 transcription factor binding sites. Fusion of the cytokine-responsive IL-8 promoter with a cDNA encoding the murine IL-4 receptor (mIL-4R) allows measurement of promoter activation by detection of the heterologous reporter protein (mIL-4R) on the cell surface of transfected cells.

Human kidney epithelial cells (293/EBNA) are transfected (via the DEAE/DEXTRAN method) with plasmids encoding: 1). the reporter/promoter construct (referred to as pIL-8rep), and 2). the cDNA(s) of interest . DNA concentrations are always kept constant by the addition of empty vector DNA. The 293/EBNA cells are plated at a density of 2.5 x 10^4 cells/ml (3 ml/ well) in a 6 well plate and incubated for two days prior to transfection. Two days after transfection, the mIL-4 receptor is detected by a radioimmunoassay (RIA) described below.

In one such experiment, the 293/EBNA cells were co-transfected with DNA encoding RANK and with DNA encoding RANKL (see Example 7 below). Co-expression of this receptor and its counterstructure by cells results in activation of the signaling process of RANK. For such co-transfection studies, the DNA concentration/well for the DEAE transfection were as follows: 40 ng of pIL-8rep [pBluescriptSK- vector (Stratagene)]; 0.4 ng CD40 (DNA encoding CD40, a control receptor; pCDM8 vector); 0.4 ng RANK (DNA encoding RANK; pDC409 vector), and either 1-50 ng CD40L (DNA encoding the ligand for CD40, which acts as a positive control when co-transfected with CD40 and as a negative control when co-transfected with RANK; in pDC304) or RANKL (DNA encoding a ligand for RANK; in pDC406). Similar experiments can be done using soluble RANKL or agonistic antibodies to RANK to trigger cells transfected with RANK.

For the mIL-4R-specific RIA, a monoclonal antibody reactive with mIL-4R is labeled with ^{125}I via a Chloramine T conjugation method; the resulting specific activity is typically 1.5 x 10^{16} cpm/nmol. After 48 hours, transfected cells are washed once with media (DMEM/F12 5% FBS). Non-specific binding sites are blocked by the addition of pre-warmed binding media containing 5% non-fat dry milk and incubation at 37°C/5% CO_2 in a tissue culture incubator for one hour. The blocking media is decanted and binding buffer containing ^{125}I anti-mIL-4R (clone M1; rat IgG1) is added to the cells and incubated with rocking at room temperature for 1 hour. After incubation of the cells with the radio-labeled antibody, cells are washed extensively with binding buffer (2X) and twice with

with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in US patent 5,599,905, issued February 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO: 12, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9.

This ligand is useful for assessing the ability of RANK to bind RANKL by a number of different assays. For example, transfected cells expressing RANKL can be used in a FACS assay (or similar assay) to evaluate the ability of soluble RANK to bind RANKL. Moreover, soluble forms of RANKL can be prepared and used in assays that are known in the art (i.e., ELISA or BIAcore assays essentially as described in USSN 08/249,189, filed May 24, 1994). RANKL is also useful in affinity purification of RANK, and as a reagent in methods to measure the levels of RANK in a sample. Soluble RANKL is also useful in inducing NF-κB activation and thus protecting cells that express RANK from apoptosis.

EXAMPLE 8

This example describes the isolation of a human RANK ligand (RANKL) using a PCR-based technique. Murine RANK ligand-specific oligonucleotide primers were used in PCR reactions using human cell line-derived first strand cDNAs as templates. Primers corresponded to nucleotides 478-497 and to the complement of nucleotides 858-878 of murine RANK ligand (SEQ ID NO:10). An amplified band approximately 400 bp in length from one reaction using the human epidermoid cell line KB (ATCC CCL-17) was gel purified, and its nucleotide sequence determined; the sequence was 85% identical to the corresponding region of murine RANK ligand, confirming that the fragment was from human RANKL.

To obtain full-length human RANKL cDNAs, two human RANKL-specific oligonucleotides derived from the KB PCR product nucleotide sequence were radiolabeled and used as hybridization probes to screen a human PBL cDNA library prepared in lambda gt10 (Stratagene, La Jolla, CA), substantially as described in US patent 5,599,905, issued February 4, 1997 . Several positive hybridizing plaques were identified and purified, their inserts subcloned into pBluescript SK- (Stratagene, La Jolla, CA), and their nucleotide sequence determined. One isolate, PBL3, was found to encode most of the predicted human RANKL, but appeared to be missing approximately 200 bp of 5' coding region. A second isolate, PBL5 was found to encode much of the predicted human RANKL, including the entire 5' end and an additional 200 bp of 5' untranslated sequence.

diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL.

To immunize rodents, RANKL immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intamuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANKL, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in US Patent 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANKL protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANKL and inhibit binding to RANK) and non-blocking (i.e., antibodies that bind RANKL and do not inhibit binding) are isolated.

EXAMPLE 12

This example illustrates the influence of RANK.Fc and hRANKL on activated T cell growth. The addition of TGFß to anti-CD3 activated human peripheral blood T lymphocytes induces proliferation arrest and ultimately death of most lymphocytes within the first few days of culture. We tested the effect of RANK:RANKL interactions on TGFß-treated T cells by adding RANK.Fc or soluble human RANKL to T cell cultures.

Human peripheral blood T cells (7 x 10^5 PBT) were cultured for six days on anti-CD3 (OKT3, 5µg/ml) and anti-Flag (M1, 5µg/ml) coated 24 well plates in the presence of TGFß (1ng/ml) and IL-4 (10ng/ml), with or without recombinant FLAG-tagged soluble hRANKL (1µg/ml) or RANK.Fc (10µg/ml). Viable T cell recovery was determined by triplicate trypan blue countings.

The addition of RANK.Fc significantly reduced the number of viable T cells recovered after six days, whereas soluble RANKL greatly increased the recovery of viable T cells (Figure 1). Thus, endogenous or exogenous RANKL enhances the number of viable T cells generated in the presence of TGFß. TGFß, along with IL-4, has been implicated in immune response regulation when secreted by the T_H3/regulatory T cell subset. These T cells are believed to mediate bystander suppression of effector T cells. Accordingly, RANK and its ligand may act in an auto/paracrine fashion to influence T cell tolerance. Moreover, TGFß is known to play a role in the evasion of the immune system effected by certain pathogenic or opportunistic organisms. In addition to playing a role in the development of tolerance, RANK may also play a role in immune system evasion by pathogens.

EXAMPLE 13

This example illustrates the influence of the interaction of RANK on CD1a+ dendritic cells (DC). Functionally mature dendritic cells (DC) were generated *in vitro* from CD34+ bone marrow (BM) progenitors. Briefly, human BM cells from normal healthy volunteers were density fractionated using Ficoll medium and CD34+ cells immunoaffinity isolated using an anti-CD34 matrix column (Ceprate, CellPro). The CD34+ BM cells were then cultured in human GM-CSF (20 ng/ml), human IL-4 (20 ng/ml), human TNF-α (20 ng/ml), human CHO-derived Flt3L (FL; 100 ng/ml) in Super McCoy's medium supplemented with 10% fetal calf serum in a fully humidified 37°C incubator (5% CO_2) for 14 days. CD1a+, HLA-DR+ DC were then sorted using a FACStar Plus™, and used for biological evaluation of RANK

On human CD1a+ DC derived from CD34+ bone marrow cells, only a subset (20-30%) of CD1a+ DC expressed RANK at the cell surface as assessed by flow cytometric

interactions, RANK and its ligand may form an important part of the activation cascade that is induced during DC-mediated T cell expansion. Furthermore, culture of DC in RANKL results in decreased levels of CD1b/c expression, and increased levels of CD83. Both of these molecules are similarly modulated during DC maturation by CD40L (Caux et al. *J. Exp. Med.* 180:1263; 1994), indicating that RANKL induces DC maturation.

Dendritic cells are referred to as "professional" antigen presenting cells, and have a high capacity for sensitizing MHC-restricted T cells. There is growing interest in using dendritic cells *ex vivo* as tumor or infectious disease vaccine adjuvants (see, for example, Romani, et al., *J. Exp. Med.*, 180:83, 1994). Therefore, an agent such as RANKL that induces DC maturation and enhances the ability of dendritic cells to stimulate an immune response is likely to be useful in immunotherapy of various diseases.

EXAMPLE 14

This example describes the isolation of the murine homolog of RANK, referred to as muRANK. MuRANK was isolated by a combination of cross-species PCR and colony hybridization. The conservation of Cys residues in the Cys-rich pseudorepeats of the extracellular domains of TNFR superfamily member proteins was exploited to design human RANK-based PCR primers to be used on murine first strand cDNAs from various sources. Both the sense upstream primer and the antisense downstream primer were designed to have their 3' ends terminate within Cys residues.

The upstream sense primer encoded nucleotides 272-295 of SEQ ID NO:5 (region encoding amino acids 79-86); the downstream antisense primer encoded the complement of nucleotides 409-427 (region encoding amino acids 124-130). Standard PCR reactions were set up and run, using these primers and first strand cDNAs from various murine cell line or tissue sources. Thirty reaction cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 20 seconds were run. PCR products were anlyzed by electrophoresis, and specific bands were seen in several samples. The band from one sample was gel purified and DNA sequencing revealed that the sequence between the primers was approximately 85% identical to the corresponding human RANK nucleotide sequence.

A plasmid based cDNA library prepared from the murine fetal liver epithelium line FLE18 (one of the cell lines identified as positive in the PCR screen) was screened for full-length RANK cDNAs using murine RANK-specific oligonucleotide probes derived from the murine RANK sequence determined from sequencing the PCR product. Two cDNAs, one encoding the 5' end and one encoding the 3' end of full-length murine RANK (based on sequence comparison with the full-length human RANK) were recombined to generate a full-length murine RANK cDNA. The nucleotide and amino acid seqeunce of muRANK are shown in SEQ ID Nos:14 and 15.

A soluble, oligomeric form of huRANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to a codon encoding Asp followed by DNA ending a trimer-former "leucine" zipper (SEQ ID NO:19), then by codons encoding Thr Arg Ser followed by amino acids 138-317 of SEQ ID NO:13.

These and other constructs are prepared by routine experimentation. The various DNAs are then inserted into a suitable expression vector, and expressed. Particularly preferred expression vectors are those which can be used in mammalian cells. For example, pDC409 and pDC304, described herein, are useful for transient expression. For stable transfection, the use of CHO cells is preferred; several useful vectors are described in USSN 08/785,150, now allowed, for example, one of the 2A5-3 λ-derived expression vectors discussed therein.

EXAMPLE 16

This example demonstrates that RANKL expression can be up-regulated on murine T cells. Cells were obtained from mesenteric lymph nodes of C57BL/6 mice, and activated with anti-CD3 coated plates, Concanavalin A (ConA) or phorbol myristate acetate in combination with ionomycin (anti-CD3: 500A2; Immunex Corporation, Seattle WA; ConA, PMA, ionomycin, Sigma, St. Louis, MO) substantially as described herein, and cultured from about 2 to 5 days. Expression of RANKL was evaluated in a three color analysis by FACS, using antibodies to the T cell markers CD4, CD8 and CD45RB, and RANK/Fc, prepared as described herein.

RANKL was not expressed on unstimulated murine T cells. T cells stimulated with either anti-CD3, ConA, or PMA/ionomycin, showed differential expression of RANKL: $CD4^+/CD45RB^{Lo}$ and $CD4^+/CD45RB^{Hi}$ cells were positive for RANKL, but CD8+ cells were not. RANKL was not observed on B cells, similar to results observed with human cells.

EXAMPLE 17

This example illustrates the effects of murine RANKL on cell proliferation and activation. Various cells or cell lines representative of cells that play a role in an immune response (murine spleen, thymus and lymphnode) were evaluated by culturing them under conditions promoting their viability, in the presence or absence of RANKL. RANKL did not stimulate any of the tested cells to proliferate. One cell line, a macrophage cell line referred to as RAW 264.7 (ATCC accession number TIB 71) exhibited some signs of activation.

RAW cells constitutively produce small amounts of TNF-α. Incubation with either human or murine RANKL enhanced production of TNF-α by these cells in a dose

Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 1 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs.

Radio-labeled (^{35}S-Met, Cys) TRAF proteins were prepared by *in vitro* translation using a commercially available reticulocyte lysate kit according to manufacturer's instructions (Promega). Truncated GST fusion proteins were purified substantially as described in Smith and Johnson (supra). Briefly, *E. coli* were transfected with an expression vector encoding a fusion protein, and induced to express the protein. The bacteria were lysed, insoluble material removed, and the fusion protein isolated by precipitation with glutathione-coated beads (Sepahrose 4B, Pharmacia, Uppsala Sweden)

The beads were washed, and incubated with various radiolabeled TRAF proteins. After incubation and wash steps, the fusion protein/TRAF complexes were removed from the beads by boiling in 0.1% SDS + ß-mercaptoethanol, and loaded onto 12% SDS gels (Novex). The gels were subjected to autoradiography, and the presence or absence of radiolabeled material recorded. The results are shown in Table 2 below.

Table 2: Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK

C terminal Truncations:	E206-S339	E206-Y421	E206-M476	E206-G544	Full length
TRAF1	-	-	-	-	++
TRAF2	-	-	-	-	++
TRAF3	-	-	-	-	++
TRAF4	-	-	-	-	-
TRAF5	-	-	-	-	+
TRAF6	-	+	+	+	++

These results indicate that TRAF1, TRAF2, TRAF3, TRAF 5 and TRAF6 bind to the most distal portion of the RANK cytoplasmic domain (between amino-acid G544 and A616). TRAF6 also has a binding site between S339 and Y421. In this experiment, TRAF5 also bound the cytoplasmic domain of RANK.

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
(B) CLONE: 9D-8A

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 93..1868

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCTGCTGCTG CTCTGCGCGC TGCTCGCCCG GCTGCAGTTT TATCCAGAAA GAGCTGTGTG    60

GACTCTCTGC CTGACCTCAG TGTTCTTTTC AG GTG GCT TTG CAG ATC GCT CCT     113
                                    Val Ala Leu Gln Ile Ala Pro
                                      1               5

CCA TGT ACC AGT GAG AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC     161
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
        10                  15                  20

AAA TGT GAA CCA GGA AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT     209
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
    25                  30                  35

GAC AGT GTA TGT CTG CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG     257
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 40                  45                  50                  55

AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG     305
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
                60                  65                  70

GCC CTG GTG GCC GTG GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC     353
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
            75                  80                  85

GCG TGC ACG GCT GGG TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC     401
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
        90                  95                  100

CGC AAC ACC GAG TGC GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG     449
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
    105                 110                 115

CTC AAC AAG GAC ACA GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT     497
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
120                 125                 130                 135
```

AGC TGC AAC TGC ACT GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG 1265
Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
380 385 390

TCC TCT GAA AAC TAC TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG 1313
Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
395 400 405

CAC TGG GCA GCC AGC CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC 1361
His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
410 415 420

TGC CGG AAC CCT CCT GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA 1409
Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
425 430 435

AAA CGT GGA CCC TTG CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT 1457
Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
440 445 450 455

GAA GAA GAA GCC AGC AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG 1505
Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
460 465 470

GCT GAT GGG AGG CTC CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA 1553
Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
475 480 485

AGC TCC CCT GGT GGC CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC 1601
Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
490 495 500

AGT AAC TCC ACG TTC ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC 1649
Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
505 510 515

GAC ATC ATC GTG GTC TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG 1697
Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
520 525 530 535

GCG GCT GCG GAG CCC ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG 1745
Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
540 545 550

CGC CGA GAC TCC TTC GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC 1793
Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
555 560 565

GGC GGC CCC GAG GGG CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG 1841
Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
570 575 580

CAG GAG CAA GGC GGG GCC AAG GCT TGA GCGCCCCCCA TGGCTGGGAG 1888
Gln Glu Gln Gly Gly Ala Lys Ala
585 590

CCCGAAGCTC GGAGCCAGGG CTCGCGAGGG CAGCACCGCA GCCTCTGCCC CAGCCCCGGC 1948

CACCCAGGGA TCGATCGGTA CAGTCGAGGA AGACCACCCG GCATTCTCTG CCCACTTTGC 2008

CTTCCAGGAA ATGGGCTTTT CAGGAAGTGA ATTGATGAGG ACTGTCCCCA TGCCCACGGA 2068

Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val Val Ala Gly Asn
65 70 75 80

Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly Tyr His Trp Ser
85 90 95

Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys Ala Pro Gly Leu
100 105 110

Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr Val Cys Lys Pro
115 120 125

Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser Thr Asp Lys Cys
130 135 140

Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg Val Glu His His
145 150 155 160

Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser Leu Pro Ala Arg
165 170 175

Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly Leu Ile Ile Leu
180 185 190

Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile Ile Phe Gly Val
195 200 205

Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn Leu Trp His Trp
210 215 220

Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys Glu Ser Ser Gly
225 230 235 240

Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly Gln Gln Gly Ala
245 250 255

Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys Thr Phe Pro Glu
260 265 270

Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln Gly Thr Cys Val
275 280 285

Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg Met Leu Ser Leu
290 295 300

Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg Gln Met Pro Thr
305 310 315 320

Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr Asp Gln Leu Leu
325 330 335

Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro Phe Ser Glu Pro
340 345 350

Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys Phe Thr Gly Thr
355 360 365

Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr Glu Pro Leu Cys
370 375 380

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC          53
                                          Met Ala Pro Arg Ala
                                            1               5

CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC      101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
            10                  15                  20

GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG      149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
        25                  30                  35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA      197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
    40                  45                  50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG      245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
55                  60                  65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA      293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
70                  75                  80                  85

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG      341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                90                  95                  100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG      389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
            105                 110                 115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC      437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
        120                 125                 130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA      485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
    135                 140                 145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC      533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150                 155                 160                 165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA      581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                170                 175                 180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT      629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
            185                 190                 195
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CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC 1391
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn
440 445 450

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
1 5 10 15

Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
20 25 30

Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
35 40 45

Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
50 55 60

Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
65 70 75 80

Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
85 90 95

Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
100 105 110

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
115 120 125

Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
130 135 140

Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145 150 155 160

Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
165 170 175

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
180 185 190

Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
195 200 205

Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
210 215 220

Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225 230 235 240

(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
(B) CLONE: FULL LENGTH RANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC          53
                                          Met Ala Pro Arg Ala
                                            1               5

CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC        101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
                10                  15                  20

GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG        149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
            25                  30                  35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA        197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
        40                  45                  50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG        245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
    55                  60                  65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA        293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
70                  75                  80                  85

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG        341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                90                  95                  100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG        389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
            105                 110                 115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC        437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
        120                 125                 130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA        485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
    135                 140                 145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC        533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150                 155                 160                 165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA        581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                170                 175                 180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT        629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
            185                 190                 195
```

```
CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT   1397
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro
        440                 445                 450

GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CGT GGA CCC TTG   1445
Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu
    455                 460                 465

CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT GAA GAA GAA GCC AGC   1493
Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser
470                 475                 480                 485

AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG CTC   1541
Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu
                490                 495                 500

CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA AGC TCC CCT GGT GGC   1589
Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly
            505                 510                 515

CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG TTC   1637
Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe
        520                 525                 530

ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC GAC ATC ATC GTG GTC   1685
Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val
    535                 540                 545

TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG GCG GCT GCG GAG CCC   1733
Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu Pro
550                 555                 560                 565

ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC TTC   1781
Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe
                570                 575                 580

GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG GGG   1829
Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly
            585                 590                 595

CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC GGG   1877
Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly
        600                 605                 610

GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCGAAG CTCGGAGCCA           1926
Ala Lys Ala
    615

GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCCAGCCCC GGCCACCCAG GGATCGATCG 1986

GTACAGTCGA GGAAGACCAC CCGGCATTCT CTGCCCACTT TGCCTTCCAG GAAATGGGCT 2046

TTTCAGGAAG TGAATTGATG AGGACTGTCC CCATGCCCAC GGATGCTCAG CAGCCCGCCG 2106

CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACTCGC AGCAGTAATT TGTGGCACTA 2166

TGACAGCTAT TTTTATGACT ATCCTGTTCT GTGGGGGGGG GGTCTATGTT TTCCCCCCAT 2226

ATTTGTATTC CTTTTCATAA CTTTTCTTGA TATCTTTCCT CCCTCTTTTT TAATGTAAAG 2286

GTTTTCTCAA AAATTCTCCT AAAGGTGAGG GTCTCTTTCT TTTCTCTTTT CCTTTTTTTT 2346
```

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
115 120 125

Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
130 135 140

Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145 150 155 160

Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
165 170 175

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
180 185 190

Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
195 200 205

Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
210 215 220

Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225 230 235 240

Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
245 250 255

Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
260 265 270

Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
275 280 285

Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
290 295 300

Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
305 310 315 320

Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
325 330 335

Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
340 345 350

Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
355 360 365

Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
370 375 380

Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
385 390 395 400

Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
405 410 415

Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
420 425 430

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1               5                  10                  15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
```

```
GCT CCG GCG CCG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC      95
Ala Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu
            20                  25                  30

CTG GGG CTG GGA CTG GGC CAG GTG GTC TGC AGC ATC GCT CTG TTC CTG     143
Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu
        35                  40                  45

TAC TTT CGA GCG CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT     191
Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr
    50                  55                  60

CAC TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GAT TTG CAG     239
His Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln
65                  70                  75

GAC TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TGC AGG AGG     287
Asp Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg
80                  85                  90                  95

ATG AAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT     335
Met Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile
            100                 105                 110

GTG GGG CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATG GAA GGC TCA     383
Val Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser
        115                 120                 125

TGG TTG GAT GTG GCC CAG CGA GGC AAG CCT GAG GCC CAG CCA TTT GCA     431
Trp Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala
    130                 135                 140

CAC CTC ACC ATC AAT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC     479
His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val
145                 150                 155

ACT CTG TCC TCT TGG TAC CAC GAT CGA GGC TGG GCC AAG ATC TCT AAC     527
Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn
160                 165                 170                 175

ATG ACG TTA AGC AAC GGA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT     575
Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr
            180                 185                 190

TAC CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC     623
Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser
        195                 200                 205

GTA CCT ACA GAC TAT CTT CAG CTG ATG GTG TAT GTC GTT AAA ACC AGC     671
Val Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser
    210                 215                 220

ATC AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA     719
Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys
225                 230                 235

AAC TGG TCG GGC AAT TCT GAA TTC CAC TTT TAT TCC ATA AAT GTT GGG     767
Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly
240                 245                 250                 255
```

```
Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln Asp
 65                  70                  75                  80

Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
                 85                  90                  95

Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
            100                 105                 110

Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
        115                 120                 125

Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
    130                 135                 140

Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
145                 150                 155                 160

Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
                165                 170                 175

Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
            180                 185                 190

Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
        195                 200                 205

Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
    210                 215                 220

Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
225                 230                 235                 240

Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
                245                 250                 255

Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
            260                 265                 270

Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
        275                 280                 285

Lys Val Gln Asp Ile Asp
    290
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 954 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

```
TGG GCC AAG ATC TCC AAC ATG ACT TTT AGC AAT GGA AAA CTA ATA GTT   624
Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
        195                 200                 205

AAT CAG GAT GGC TTT TAT TAC CTG TAT GCC AAC ATT TGC TTT CGA CAT   672
Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
    210                 215                 220

CAT GAA ACT TCA GGA GAC CTA GCT ACA GAG TAT CTT CAA CTA ATG GTG   720
His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
225                 230                 235                 240

TAC GTC ACT AAA ACC AGC ATC AAA ATC CCA AGT TCT CAT ACC CTG ATG   768
Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
                245                 250                 255

AAA GGA GGA AGC ACC AAG TAT TGG TCA GGG AAT TCT GAA TTC CAT TTT   816
Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
            260                 265                 270

TAT TCC ATA AAC GTT GGT GGA TTT TTT AAG TTA CGG TCT GGA GAG GAA   864
Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
        275                 280                 285

ATC AGC ATC GAG GTC TCC AAC CCC TCC TTA CTG GAT CCG GAT CAG GAT   912
Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
    290                 295                 300

GCA ACA TAC TTT GGG GCT TTT AAA GTT CGA GAT ATA GAT TGA           954
Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
305                 310                 315
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 317 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu
 1               5                  10                  15

Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala
            20                  25                  30

Pro Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met
        35                  40                  45

Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val
    50                  55                  60

Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser
65                  70                  75                  80

Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn
                85                  90                  95
```

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Murine Fetal Liver Epithelium
(B) CLONE: muRANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
ATG GCC CCG CGC GCC CGG CGG CGC CGC CAG CTG CCC GCG CCG CTG CTG   48
Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
 1               5                  10                  15

GCG CTC TGC GTG CTG CTC GTT CCA CTG CAG GTG ACT CTC CAG GTC ACT   96
Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
            20                  25                  30

CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC  144
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
        35                  40                  45

AGC AGA TGC GAA CCA GGA AAG TAC CTG TCC TCT AAG TGC ACT CCT ACC  192
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
    50                  55                  60

TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC  240
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65                  70                  75                  80

TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC  288
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
                85                  90                  95

AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC CAC ACG GCC CCG CGT CGC  336
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
            100                 105                 110

TGT GCT TGC ACG GCT GGC TAC CAC TGG AAC TCA GAC TGC GAG TGC TGC  384
Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
        115                 120                 125

CGC AGG AAC ACG GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG  432
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
    130                 135                 140

CAG CTC AAC AAG GAT ACG GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC  480
Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145                 150                 155                 160

TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC  528
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
                165                 170                 175

ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT  576
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
            180                 185                 190
```

```
AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG  1344
Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
        435                 440                 445

GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC  1392
Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
    450                 455                 460

CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG  1440
Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465                 470                 475                 480

GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC  1488
Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
                485                 490                 495

TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC  1536
Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
            500                 505                 510

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG  1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
        515                 520                 525

AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG  1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
    530                 535                 540

GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT  1680
Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545                 550                 555                 560

GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG  1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
                565                 570                 575

CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG  1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
            580                 585                 590

GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG  1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
        595                 600                 605

GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA  1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
    610                 615                 620

                                                                1878
GAA TGA
Glu
625
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
305 310 315 320

Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
325 330 335

Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
340 345 350

Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
355 360 365

Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
370 375 380

Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
385 390 395 400

Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
405 410 415

His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
420 425 430

Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
435 440 445

Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
450 455 460

Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465 470 475 480

Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
485 490 495

Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
500 505 510

Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
515 520 525

Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
530 535 540

Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545 550 555 560

Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
565 570 575

Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
580 585 590

Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
595 600 605

Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
610 615 620

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
1 5 10 15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
20 25 30

Arg

8. A recombinant expression vector comprising a DNA sequence according to claim 2.

9. A recombinant expression vector comprising a DNA sequence according to claim 3.

10. A recombinant expression vector comprising a DNA sequence according to claim 4.

11. A recombinant expression vector comprising a DNA sequence according to claim 5.

12. A recombinant expression vector comprising a DNA sequence according to claim 6.

13. A host cell transformed or transfected with an expression vector according to claim 7.

14. A host cell transformed or transfected with an expression vector according to claim 8.

15. A host cell transformed or transfected with an expression vector according to claim 9.

16. A host cell transformed or transfected with an expression vector according to claim 10.

17. A host cell transformed or transfected with an expression vector according to claim 11.

18. A host cell transformed or transfected with an expression vector according to claim 12.

19. A process for preparing a RANK protein, comprising culturing a host cell according to claim 13 under conditions promoting expression and recovering the RANK .

20. A process for preparing a RANK protein, comprising culturing a host cell according to claim 14 under conditions promoting expression and recovering the RANK .

Day 6, viable T cell recovery (x10-3/ml)
0
25
50
75
100
Medium
RANK-Fc
RANK-L

Figure 1

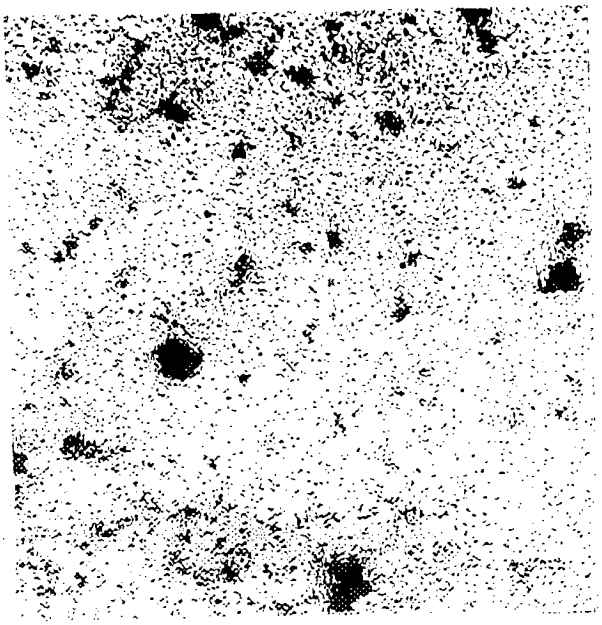

Fig. 2A

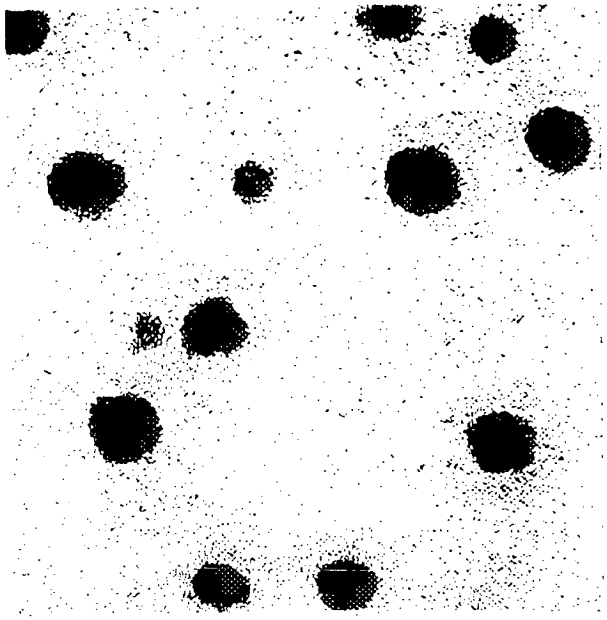

Fig. 2B

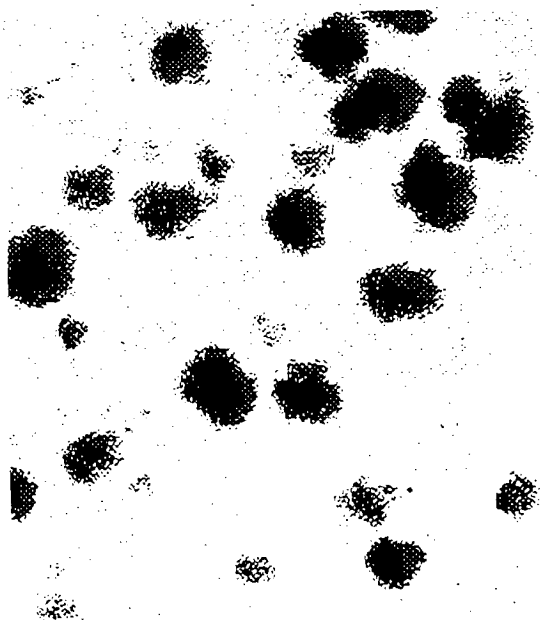

Fig. 2C

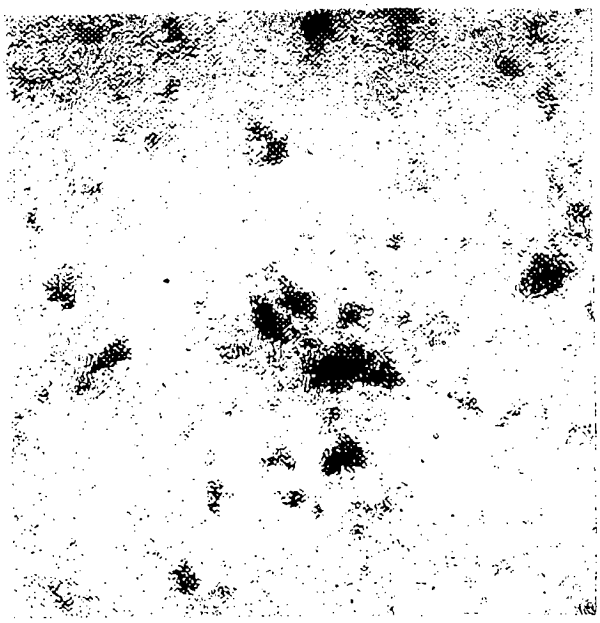

Fig. 2D

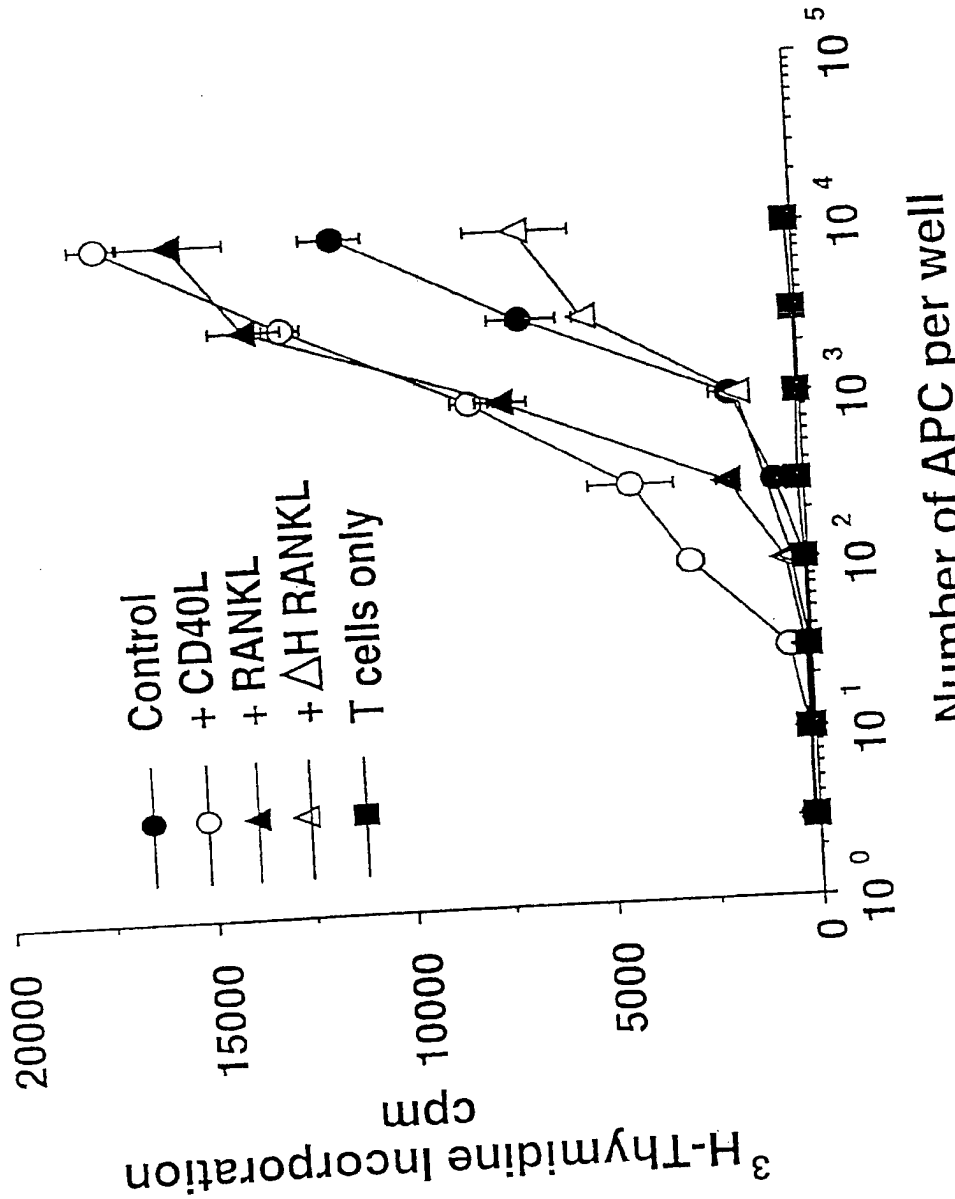
^{3}H-Thymidine Incorporation
cpm
20000
15000
10000
5000
0
Control
+ CD40L
+ RANKL
+ ΔH RANKL
T cells only
10^0
10^1
10^2
10^3
10^4
10^5
Number of APC per well

Figure 3

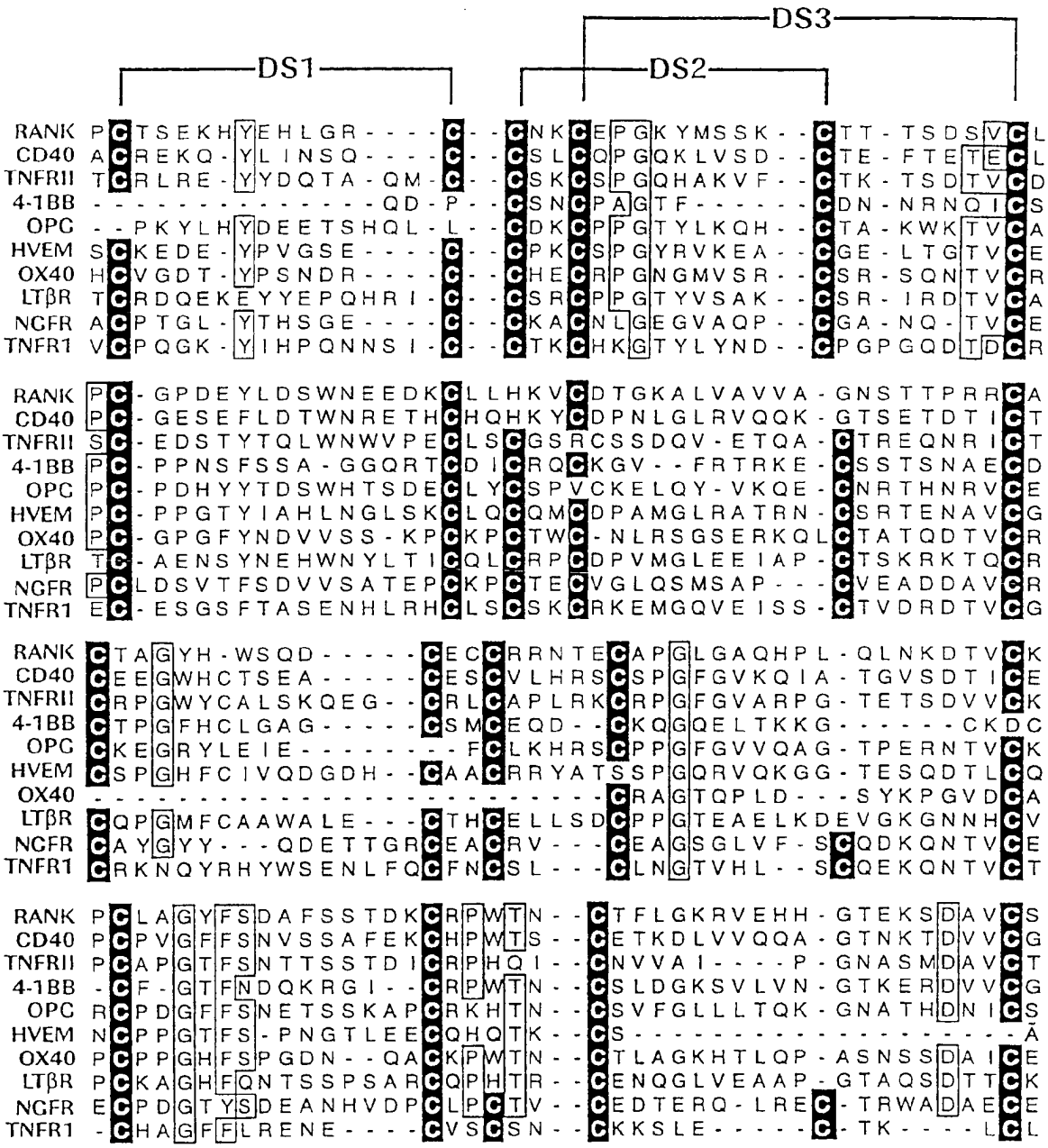
DS1
DS2
DS3
RANK
CD40
TNFRII
4-1BB
OPG
HVEM
OX40
LTβR
NGFR
TNFR1

Figure 4

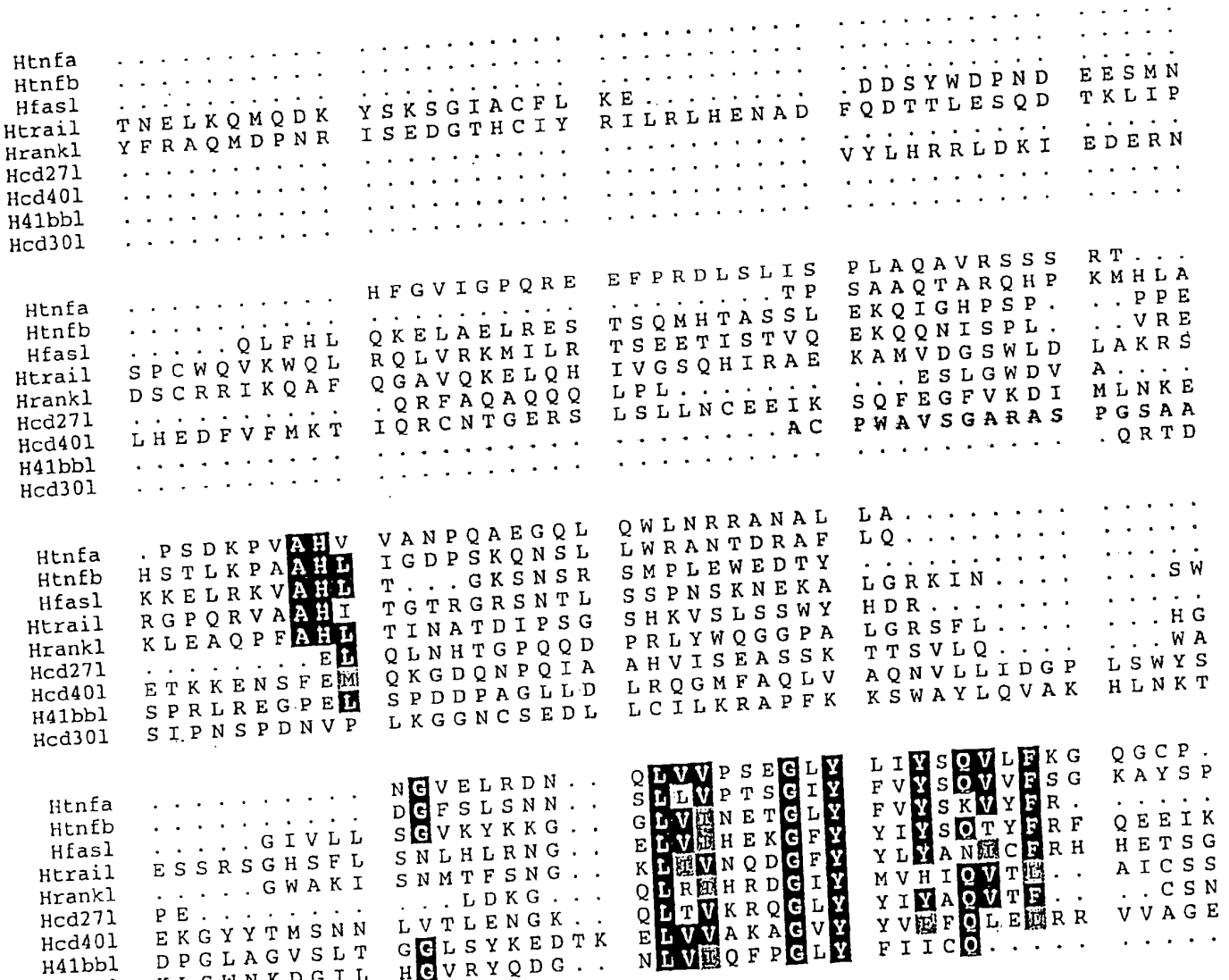
Htnfa
Htnfb
Hfasl
Htrail
Hrankl
Hcd27l
Hcd40l
H41bbl
Hcd30l

Figure 5

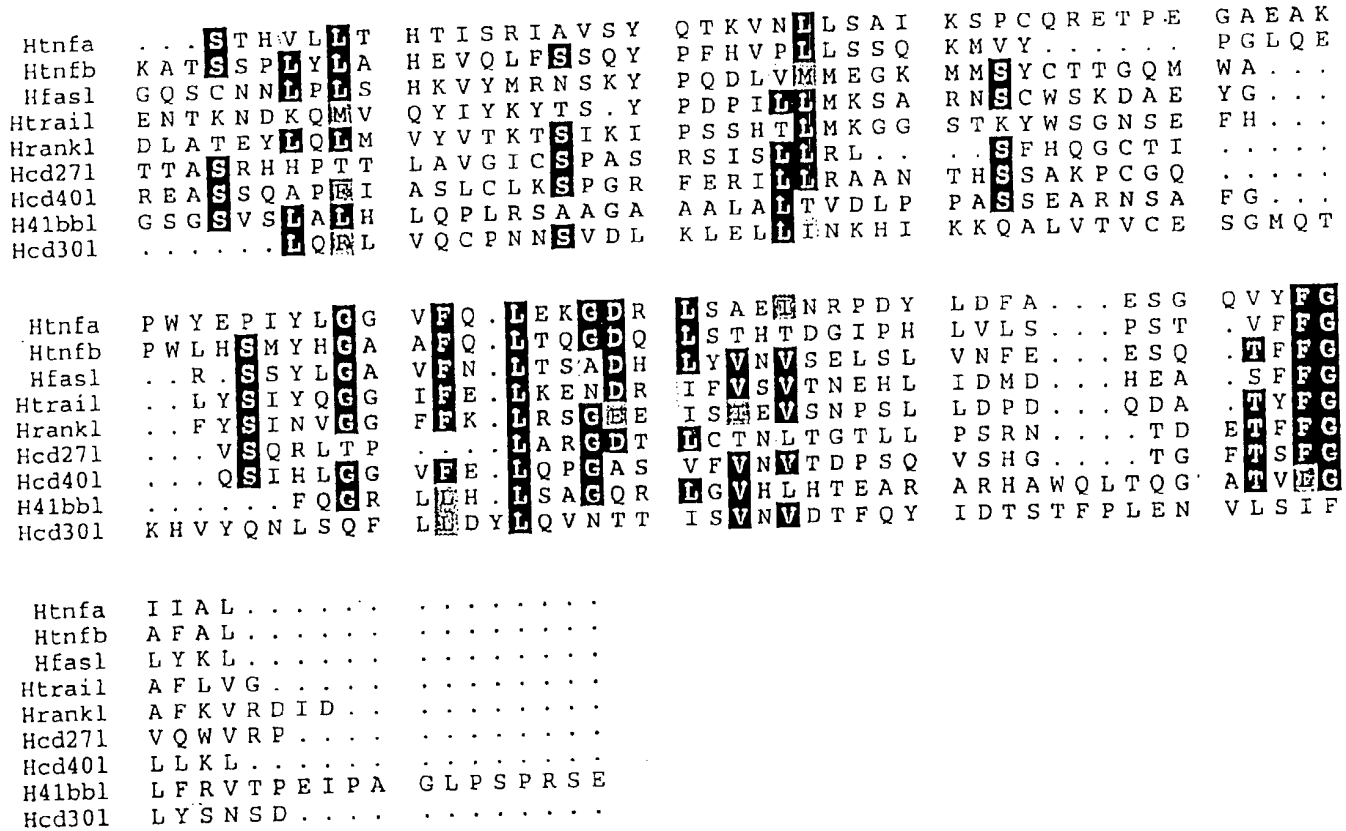

Figure 5 (cont.)